

Assessment of Heavy Metal Pollution in *Octopus cyanea* in the Coastal Waters of Tanzania

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Introduction

The accumulation of toxic metals, to hazardous levels, in marine environments has become a problem of increasing concern. Coastal waters bordering urban areas receive significant anthropogenic input from various point and non-point sources, such as municipal areas, tourism and industries. The Western Indian Ocean (WIO) region, which includes the coastal waters of Tanzania, are fed by several rivers and streams and receive a substantial quantity of pollutant-containing sedimentary material. In Tanzania, for example, large amounts of industrial and domestic wastes are released without undergoing prior treatment.¹ It is estimated that municipal waste generated in the city of Dar es Salaam alone amounts to 1,770 tonnes per day.² However, only 8.1% of this

Background. The accumulation of toxic metals in marine environments has become a problem of increasing concern. In Tanzania, large amounts of industrial and domestic waste from major coastal cities such as Dar es Salaam, Tanga and Zanzibar are released into the marine environment without undergoing treatment. The wastes are sometimes contaminated with hazardous heavy metals such as lead, zinc and cadmium, among others. Elevated concentrations of these metals have been measured in sediment along the coastal waters of Tanzania. These metals have effects on both aquatic organisms and humans through consumption of contaminated fish and other aquatic foods. Despite the social-economic support provided by marine biota, there is no information on the levels and magnitude of octopus contamination by heavy metals or their safety for human consumption.

Objective. Investigate the spatial concentrations of lead in the muscle tissue and liver of *O. cyanea* and sediment in the coastal waters off Dar es Salaam and Tanga, Tanzania.

Methods. Concentrations of lead, from samples of *O. cyanea* muscle and liver tissue and sediment, were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Results. The concentration of lead in the muscles and liver of *O. cyanea* ranged from 0.01 to 21.6±2.41 µg/g. On the other hand, the concentration of lead in sediment ranged from 6.33±0.39 to 13.85±3.31 µg/g.

Discussion. Levels of lead were detected in the muscle and liver tissues of the *O. cyanea*, but presumably, below toxic risk if provisional tolerable weekly intake (PTWI) levels are considered. However, the present results should be taken as worrisome in view of health implications for populations that depend on octopus as a source of protein. Sediment samples were below both Florida's 'No effect level' and the threshold value of Environment Canada.

Competing Interests. The authors declare no competing financial interests.

Keywords. *O. cyanea*, liver, muscles, sediments, lead, concentrations, Tanzania, Provisional Tolerable Weekly Intake, PTWI, World Health Organization, WHO, Food and Agricultural Organization, FAO

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is disposed of in the official dumpsite while the rest is discarded on vacant land near residential areas, or dumped by roadsides or in streams, eventually finding its way to the ocean. Even the sewage from the central sewerage system is discharged untreated into the Indian Ocean. Furthermore, Mgana and the Dar es Salaam city council reported that nearly 1,588 tonnes per year of hazardous sludge are generated by several industrial companies such as Galco, Karibu Textiles, Cottex Spinning, and Shelys Limited, among others.³ The sludge is dumped untreated on site and transported to the ocean via rivers and/or streams. These wastes contain

hazardous heavy metals such as lead, cadmium and zinc, all of which can produce a range of toxic effects in aquatic ecosystems and on human health.²

Background

Recently, elevated concentrations of metals such as lead, zinc, copper, nickel, cadmium and manganese have been detected in the sediments along the coastal waters of Tanzania.^{1,4,5} It is known that sediment acts as a reservoir for many pollutants including heavy metals, and has been used to indicate the magnitude and distribution of lead contamination in

aquatic environments.⁶ Furthermore, predatory cephalopods such as *O. cyanea*, being positioned at the top of many marine food webs, are likely to be exposed to high levels of heavy metal contamination. For example, the contamination of octopuses by metals such lead, mercury, zinc, copper, cadmium and iron has been studied by Hoda et al. in the Mediterranean, Miramand in the English Channel as well as Sonia et al. along the Portuguese coast.⁷⁻⁹ It has been reported that most aquatic organisms have the capacity to accumulate heavy metals up to 105 times the levels present in their surrounding environment.¹⁰ As a result, humans may be contaminated by organic and inorganic compounds associated with an aquatic system by the consumption of polluted fish and other aquatic foods.¹¹ Metals such as lead are widely known to be toxic to many systems of the human body. In the WIO region, local residents commonly harvest a variety of algae, mollusks, crustaceans, sea cucumbers and fish for sale and home consumption. Because marine resources provide a major source of protein for the coastal people in the region, and as fishing is still an important commercial and recreational activity, it is important to document health risks associated with consuming contaminated fish and other seafood.

Objectives

In Tanzania, octopus fisheries have increased in recent years due to population growth, tourism, and the expansion of processing industries and exportation. Consumption of octopus is also high because of local beliefs of the species' medicinal effects as an aphrodisiac. However, there has been no attempt to investigate the levels and the magnitude of heavy metal contamination of *O. cyanea* and its safety for human consumption. The objective of this study is to investigate the spatial concentrations of lead in the

Abbreviations			
<i>CBR</i>	Community Bureau of Reference	<i>m/s</i>	Meters per second
<i>EPA</i>	Environmental Protection Agency	<i>PTWI</i>	Provisional Tolerable Weekly Intake
<i>FAO</i>	Food and Agricultural Organization	<i>SEAMIC</i>	Southern and Eastern African Mineral Centre
<i>g</i>	Grams	<i>UNEP</i>	United Nations Environmental Programme
<i>HN03</i>	Nitric acid	<i>WHO</i>	World Health Organization
<i>ICP-AES</i>	Inductively coupled plasma atomic emission spectroscopy	<i>WIO</i>	Western Indian Ocean
<i>m</i>	Meters	<i>μg/g</i>	Microgram per gram
		<i>WHO</i>	World Health Organization

tissues (liver and muscle) of *O. cyanea* and sediments collected from the coastal waters of Dar es Salaam and Tanga, Tanzania as well as the associated health implications on the local communities.

Methods

Sampling Sites

The study was conducted during the rainy season from April–July 2013 at 2 coastal sites in Tanzania, namely Dar es Salaam and Tanga (Fig. 1). At each study site, 15 stations and 2 reference stations were selected for sampling. Samples of both *O. cyanea* and sediment were collected at each station. These coastal areas are very favorable for *O. cyanea* fisheries.

The two coastal cities are urbanized and possess industrial sectors. In Dar es Salaam, the study was conducted in areas located between Mbegani and the Dar es Salaam harbor. In Tanga, the study was conducted in areas between the Tanga port to Mwamba Nyama and Mwamba Wamba. Both areas are characterized by seasonal variations in water circulation

connected with the periods of northeast and southeast monsoon seasons. The southeast monsoon, which begins in April and ends in September, is usually associated with strong predominantly northerly flowing currents with speeds of up to 2 m/s. The northeast monsoon runs from November to March and is associated with weaker current speeds (>0.5 m/s). The climate is tropical, with hot weather throughout the year ranging from 26°–35°C and there are two rainy seasons: long rains from March–May and short rains from November–December. In Dar es Salaam and Tanga there are a wide variety of light industries that manufacture goods for the local and foreign markets. These include distilleries, breweries, manufacturers of cigarettes, cement, paints, pharmaceuticals, plastic, metal products, steel, chemicals, confectionery, food products, timber and wood products, petroleum products, dairy products, batteries, printing, publishing and paper products, among others. Other activities conducted in the study area include urban agriculture.

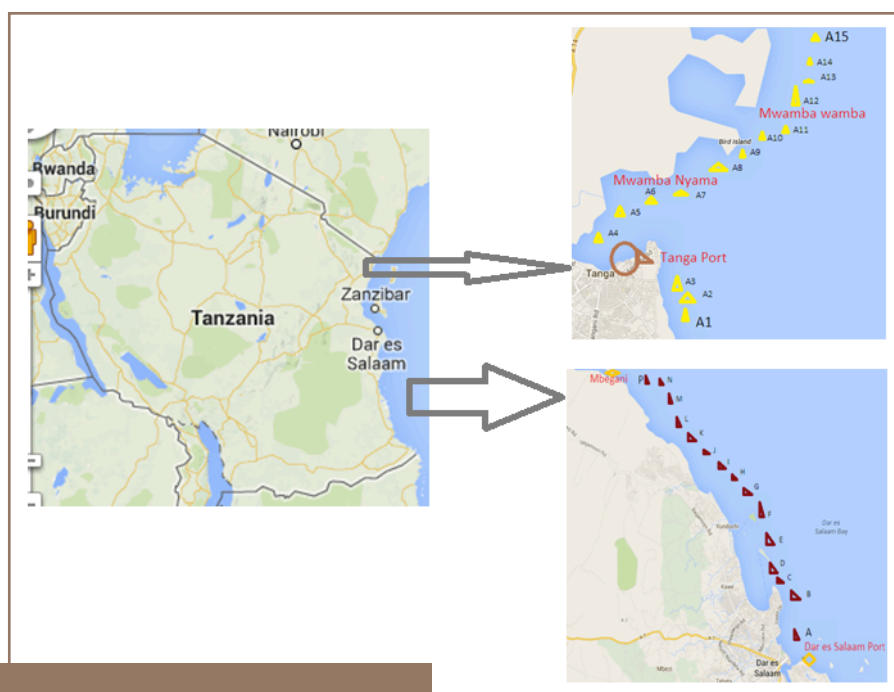


Figure 1 — Sampling Site Locations

Biological Data Sampling

A total of 60 samples (taken over 2 sampling trips) of octopuses were collected. Octopus samples were stored in pre-cleaned, acid-washed plastic containers placed in an ice chest with ice at $<0.4^{\circ}\text{C}$, then transported to the University of Dar es Salaam laboratory and stored at -20°C until analysis. In the laboratory, samples were allowed to thaw at room temperature. Octopuses were dissected to remove soft muscle and liver tissues to obtain a total of 60 samples. Sub-samples of about 10 g were taken and digested with 70% nitric acid (HN03) in a Teflon cup at $105 \pm 20^{\circ}\text{C}$ for 8 hours and frozen before analysis. It was hypothesized that concentrations of most heavy metals in octopuses may be influenced by their body weight/size. As a result, efforts were made to collect samples of nearly uniform size (400-500 g). Also, in most of the available literature, results were presented as dry weight, thus the dry weight of octopus and sediment from each station were also determined. Dry weight of the muscle tissue samples were

recorded, and the weights ranged from 0.367-0.354 and 0.476-0.482 for the tissue from Tanga and Dar es Salaam, respectively.

Environmental Data Sampling

Surface sediment samples were collected at the same locations as the octopuses. A total of 60 sediment samples weighing about 100 g each were collected from a depth of about 8m using sediment corers. The sediment samples were placed in pre-labeled transparent polythene bottles rinsed with 10.0% HN03 and kept in an ice chest at $<4^{\circ}\text{C}$. Sediments were wet-sieved through a $100\mu\text{m}$ -sized plastic screen, and approximately 2 g sub-samples (because lead tends to collect in smaller particles) of the wet sediment digested in the same way as the octopus samples. Dry weights of the sediments were 0.719 to 0.725, and 0.712 to 0.734, in Dar es Salaam and Tanga, respectively.

Laboratory Analysis

O. cyanea dried liver and muscle tissue and sediment samples were taken to the SEAMIC chemistry laboratory in

Dar es Salaam for total lead analysis. Powdered *O. cyanea* tissues (0.5 g) and frozen sediments (1.0 g) were digested for analysis. Total lead concentration in octopus tissue and sediments were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) according to the procedure described by the United Nations Environmental Programme (UNEP).¹² Both international standards from the Community Bureau of Reference (CBR) were used to check the precision and accuracy of the analysis.

Data Analysis

Data analysis was conducted with InStat (GraphPad, La Jolla, U.S.A.). The paired sample t-test was used to test for differences in the means of lead concentrations from each site. The relationships between lead concentrations in the muscles and liver were assessed using the Pearson correlation. In addition, the association between levels of lead in the liver and/or muscles of *O. cyanea* with levels in sediment was determined using the Pearson correlation. Tables and graphs were plotted to determine variations in the levels of lead pollution within and between sites. Values presented in figures are the averages of 2 samplings at each station. A probability value of $p < 0.05$ was considered as statistically significant. To assess the degree of risks that might be caused by consuming octopus contaminated with lead, the present results were compared with the permissible limits of the Food and Agriculture Organization (FAO) and the U.S. Environmental Protection Agency (EPA).¹³⁻¹⁵ Sediment samples were compared with the allowable criteria of both Florida's 'No effect level' and the 'threshold effect level' of Environment Canada.^{16,17}

Results

The spatial distribution of total lead concentrations ($\mu\text{g/g}$) in the muscle and liver of *O. cyanea* are presented in Fig 2a

and 2b, respectively. The present results were compared with the allowable limits of international standards in Table 1. The concentration of lead in the muscles, as observed in Dar es Salaam, ranged between $2.71 \pm 0.003 \mu\text{g/g}$ at station M to $7.22 \pm 1.02 \mu\text{g/g}$ at station A (Fig. 2a). In Tanga, concentration of lead ranged from $0.01 \mu\text{g/g}$ to $3.24 \pm 1.03 \mu\text{g/g}$ at station A10 and A5, respectively (Fig. 2b). The average lead concentration in the muscles of *O. cyanea* was $3.63 \mu\text{g/g}$ and $2.51 \mu\text{g/g}$ wet weight for the samples collected in Dar es Salaam and Tanga, respectively. Analysis of 2 sample t-tests for lead contamination levels in muscles was higher in Dar es Salaam than the concentration measured in Tanga ($p < 0.05$). This shows that *O. cyanea* collected from Dar es Salaam had higher lead concentrations than specimens collected from Tanga. The presence of lead in the muscles of *O. cyanea* indicates a contaminated environment in Tanzania coastal waters.

On the other hand, lead concentrations were determined in the liver of octopus collected in Dar es Salaam where the concentration ranged from $8.11 \pm 1.12 \mu\text{g/g}$ at station I to $21.6 \pm 2.41 \mu\text{g/g}$ at station C (Fig. 2a). Conversely, lead concentration in the octopus liver from Tanga ranged from $5.05 \pm 1.02 \mu\text{g/g}$ at station A10 to $10.71 \pm 1.53 \mu\text{g/g}$ at station A2 (Fig. 2b). The data shows a higher concentration of lead for the liver samples in Dar es Salaam than in Tanga ($p < 0.05$). The results suggest higher concentrations of lead in specimens of *O. cyanea* from Dar es Salaam than those from Tanga. Of interest to this study is that the concentration of lead in the liver was higher than the concentration in muscle tissues ($p < 0.05$).

Furthermore, concentrations of lead in the muscles correlated positively with the concentration of the same element in the liver ($r = 0.89$, $p < 0.05$). That is, individuals with a higher concentration of lead in the liver also had higher

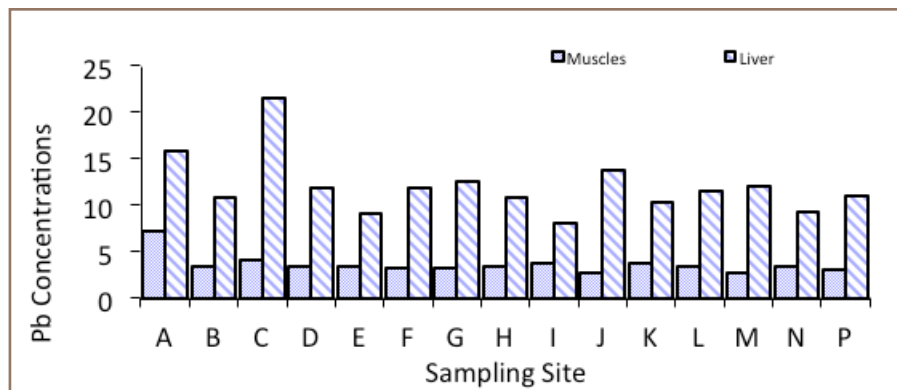


Figure 2a — Concentration of Lead ($\mu\text{g/g}$) in the muscles and liver of *O. cyanea* collected in Dar es Salaam

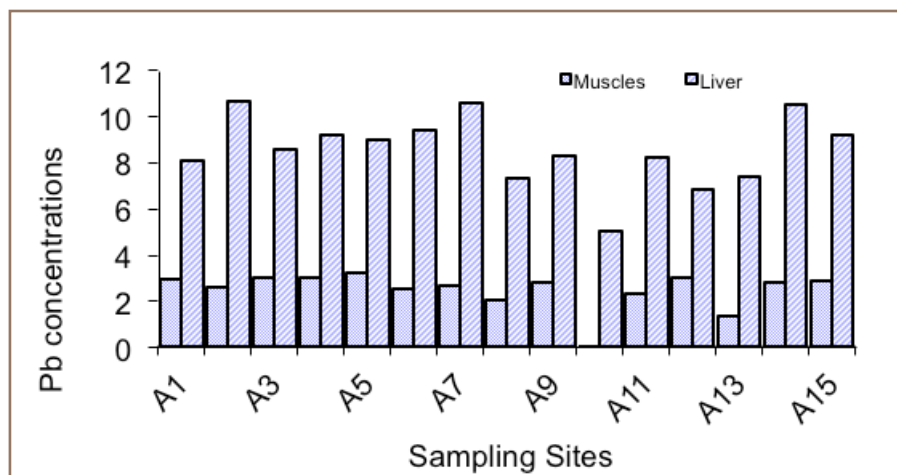


Figure 2b — Concentration of Lead ($\mu\text{g/g}$) in the muscles and liver of *O. cyanea* collected in Tanga

	mg/kg	Reference
UNEP	0.3	UNEP, 1985
IAEA-407	0.12	Wyse et al.2003
TFC	0.2	TFC, 2002
USEPA	0.05	USEPA, 1986
Directive 2005/78/EC	0.2	EC, 2005
FAO/WHO	0.2*	FAO/WHO, 2004,2006

Table 1 — The Tolerable Values of Lead (Pb) in Fish (mg/kg)

*unit given in $\mu\text{g/g}$

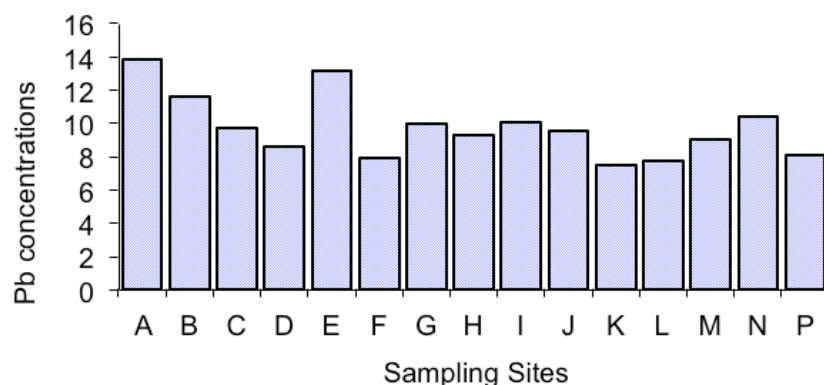


Figure 3a — Concentration of Lead (µg/g) in the sediments collected in Dar es Salaam

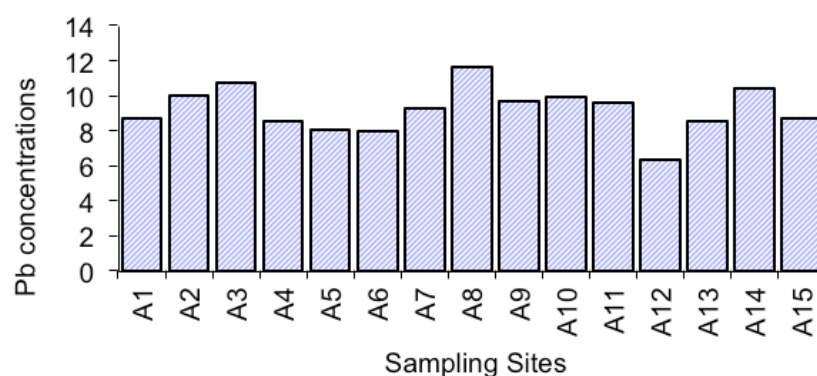


Figure 3b — Concentration of Lead (µg/g) in the sediments collected in Tanga

concentrations in muscle tissue.

Lead concentrations in the sediment samples are presented in Fig 3a and 3b. Sediments obtained in Dar es Salaam at station A revealed the highest levels of lead (13.85 ± 3.31 µg/g) while the lowest value was recorded in station K (7.48 ± 1.401 µg/g) (Fig 3a). The concentration of lead measured in Tanga ranged from 6.33 ± 0.39 µg/g at station A12 to 11.67 ± 0.85 µg/g at station A8 (Fig. 3b). Samples from reference stations also demonstrated high lead concentrations of 6.82 and 8.17 µg/g in Tanga and Dar

es Salaam, respectively. Analysis of lead in the sediment found no statistical difference between sites ($p > 0.05$). Analysis showed that concentration of lead in the sediment at Dar es Salaam correlated positively with the concentration of the same metal measured in the muscles of *O. cyanea* ($R = 0.579$, $p < 0.05$) and liver ($R = 0.89$, $p < 0.05$). That is, lead contaminants in the sediments corresponded to the concentration observed in the liver and muscle tissues. On the other hand, the concentration of lead in the sediments collected in Tanga did not correlate with

the concentration of the same elements in the muscles ($R = -0.25$, $p > 0.05$) and liver ($R = 0.069$, $p > 0.05$) of *O. cyanea*. This is an indication that increasing the concentration of lead in the sediment did not result in an increase in the concentration of the same element in the muscles and liver.

Discussion

There was a significantly higher level of lead in the liver than in the muscles of *O. cyanea*. In addition, the study found a positive correlation between the concentration of lead in the sediments and tissues of the same species in Dar es Salaam. It is known that the accumulation of lead in tissues of aquatic organisms depends on the concentration of the element in water and sediments, age of the organism, species, pH and water temperature, among other factors.¹⁸ Thus, different concentrations of lead in the muscle and liver tissues of octopus may be caused by one or more of these factors.

The feeding habits of the octopus may also contribute to the elevated concentration of lead in the muscles and liver. Being at the top of the local food chain, they feed on various food items such as mollusks, crabs and other crustaceans that spend much of their life cycle in contaminated sediment as observed in the present study. The elevated levels of lead in the liver when compared to those in the muscles could be attributed to the fact that the liver plays a crucial function in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions.¹⁹ Further studies on this heavy metal have shown a tendency to increase or decrease hepatic enzyme activities; it can cause histopathological hepatic variations, a phenomenon which depends on the type of metal and its concentration, fish species, length of exposure and other factors such as

pH and water temperature. Therefore, higher levels of lead in the liver, observed in this study, are probably an indication that metabolism of lead in octopus affects hepatic enzymes despite the fact that it does not have a tendency to bind in metallothioneins (metal binding proteins) as reported in fish and other vertebrates.²⁰

O. cyanea is a very popular seafood in the WIO region because local communities believe in the species' medicinal effects as an aphrodisiac. Therefore, its safety for human consumption is of public concern. As a consequence, to assess the safety of octopus harvested from Dar es Salaam and Tanga, the present study adopted the standard criteria for lead in fish and seafood as documented in a codex established jointly by the FAO and WHO.^{13,14} The codex utilizes the average value of lead in octopus muscle and liver tissues, which are 2.51 µg/g (for muscles) and 3.63 µg/g (for liver) for the samples collected in Tanga. The codex also utilizes the average values of 8.58 (for muscles) and 12.04 µg/g (for liver) of the octopus samples collected from Dar es Salaam waters.

These values were above the maximum allowable criteria of 0.20 µg/g wet weight in fish muscles.^{13,14} Also, these values were found to be above the tolerance limits set by the United Nation Environmental Program (UNEP), the International Atomic Energy Agency and the EPA (Table 1). That is, octopus collected from the coastal waters of Dar es Salaam and Tanga are unsafe for human consumption according to these standards.

However, we compared the same results to the EPA's Provisional Tolerable Weekly Intake (PTWI) standard of 25 µg/kg body weight. By applying the EPA recommendation of 340 g of fish per week as a consumption estimate, as well as 70 kg as a general adult human body weight, and the average lead

concentration measured in the course of this study (3.63 µg/g for Dar es Salaam and 2.51 µg/g wet weight in Tanga), consumption of *O. cyanea* muscles would fall out to a weekly intake of 17.63 µg/kg in Dar es Salaam ($3.63 \mu\text{g/g} \times 340 \text{ g}/70 \text{ kg}$ body weight) and 12.19 µg/kg in Tanga ($2.51 \mu\text{g/g} \times 340 \text{ g}/70 \text{ kg}$).¹⁵ Considering the average concentrations of lead obtained in the present study, a weekly intake of 12.19 µg/kg and 17.63 µg/kg for *O. cyanea* muscles from the coastal waters of Tanga and Dar es Salaam can be safely consumed. A weekly intake limit of lead was not calculated for octopus liver tissue because coastal communities do not consume this directly, but current harvesting and processing methods may lead to contamination of muscles through contact with contents of ruptured livers. As a result, higher concentrations of lead in the liver may lead to elevated concentrations of the same element in muscle tissue. It should be noted that the effect of harvesting and processing methods on the post-harvest differential accumulation of lead in *O. cyanea* was beyond the scope of this study.

On the basis of the present results, Tanzania coastal communities are likely to be at only minimal danger of lead exposure from ingesting *O. cyanea* muscle tissue, provided that the EPA's PTWI guidelines are followed. However, the risk of lead exposure could be increased by the cultural beliefs of coastal inhabitants (especially men) that encourage consumption of octopus. Also, the risk could be higher than expected due to the fact that seafood provides more than 95% of animal protein consumed in the coastal communities of Tanzania.²¹ It should also be taken into consideration that lead has been reported to cause hematological and gastrointestinal dysfunction, inhibit enzymes, alter cellular calcium metabolism and slow nerve conduction, and neurological dysfunction in animals. As a result, prolonged exposure to lead

contamination may consequently result in chronic nephropathy, hypertension and reproductive impairment.²²

It is important to understand that even if the levels of lead in octopus muscle tissue do not exceed the permissible limits of the PTWI, it can have significant impact on the octopuses themselves and their predators. This is because lead is known for its neurotoxic characteristics that cause behavioral deficits in most vertebrates which may result in decreasing growth rates, survival and metabolism.²³⁻²⁵ For example, Johnson reported intestinal abnormalities in *Salmo gairdineri*, or rainbow trout (currently known as *Oncorhynchus mykiss*), with lead levels of 5.93 µg/g dry weight in the liver.²¹ On the other hand, Cogun and Kargin reported minimal mortality were noted in *Oreochromis niloticus*, or Nile tilapia, with liver levels of 200-1,000 µg/g (dry weight).²⁶ Also, studies have shown that dietary levels as low as 0.1-0.5 µg/g (wet weight) are associated with learning deficits in some vertebrates.^{7,24} As is the case in the present study, the concentration of lead in the liver and muscle tissues and sediments averaged above this range, suggesting that in some areas along the Tanzania coast, predatory fish and other marine organisms feeding on *O. cyanea* might be impacted by levels of lead. Furthermore, levels of lead in the muscles of *O. cyanea* were above the levels of the same element in Octopus spp (0.8-2.7 µg/g) studied in the coastal waters of the Mediterranean Sea.⁷ A study by Denton et al. observed the concentration of 1.0 µg/g in Octopus spp caught in the coastal waters of Japan.²⁷ This value was below the concentration of the same element in the present study.

The coastal waters of Dar es Salaam and Tanga showed a detectable concentration of lead in the sediments with no clear trend from point A in the south near to

the harbor to the north at station P, which is close to Mbegani. Though tracing the source of lead in the sediments was beyond the scope of this study, previous research findings have reported a notable amount of the metal being transported in particulate form in river and stream waters.^{1,2,5} Generally, the concentration of lead in sediment was below Florida's 'No effect level' of 21 µg/g and threshold the 'effect level' of 30.2 µg/g of Environment Canada. However, the present results suggest that the large numbers of industries with untreated wastes and agricultural operations located in Dar es Salaam and Tanga might be potential sources of this hazardous metal. Apart from industries, boating and shipping activities may also contribute to the observed lead concentrations.

The study found that the concentration of lead in the sediments collected in Dar es Salaam correlated with the concentration of the same elements in the muscles and liver of *O. cyanea* while there was no correlation for the samples collected in Tanga. It is clearly known that bioaccumulation of metals in marine organisms depends not only on environmental concentrations, but also on a range of biological and environmental factors.²⁸ Lack of correlation between sediment and liver/muscles tissues in Tanga can possibly be attributed to time of exposure, prey food as well as physicochemical parameters of the water. Although octopuses are generalist feeders, in some cases they select prey species of the benthic environment on the basis of availability and size.²⁹⁻³¹ Some studies have shown that crabs are one of the most preferred foods; it is known that crabs spend considerable time in the substrate and likely absorb pollutants from the sediments. Different species have different uptake rates of heavy metals from the environment. As a result, differential uptake of lead by the prey of octopuses from the environment could probably cause

disparity between two geographical sites. This could probably be the reason for the lack of correlation between the sediments and liver/muscle tissues in Tanga. Moreover, despite the fact that biological and environmental factors were beyond the scope of this study, previous studies by Byrne et al. and Blust et al. have shown that temperature affects metal chemistry by changing chemical speciation, pH, solubility, reaction rates or physical kinetics.^{32,33} Further studies revealed that chemical speciation indicates that increase in water temperature commonly results in increase in the concentrations and activities of bioavailable metal forms, thus, enhancing metal uptake.

Conclusion

The present results show that levels of lead are detected at elevated concentrations in the liver when compared to that of the muscles of the *O. cyanea*, but below toxic risk level if PTWI is considered. However, the present results should be taken as worrisome in view of the health implications for populations that depend on octopus as a major source of protein. There was no statistical difference in the concentration of lead in the sediments collected in Dar es Salaam and Tanga. Moreover, the concentration of lead in the sediment was below both Florida's 'No effect level' and the threshold value of Environment Canada.

Monitoring of heavy metals such as lead in the coastal waters of Tanzania and the WIO region in general is recommended in view of the possible risks to the health of consumers and the ecosystem. Concentrations of lead in octopus may not currently constitute a health hazard to humans, but people should eat a diverse selection of octopus and other seafood to avoid consuming unhealthy quantities of the metal.

It is recommended that harvesting and processing methods of *O. cyanea* be assessed in view of the possible cross contamination of muscle tissues by lead-contaminated livers. Governmental and non-governmental organizations should stress community environmental education to help people understand that the dose of toxic metal that one obtains from eating octopus may depend on the quantity consumed.

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